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Research note

Anti-inflammatory and mycobacterial activity of leaf extracts of *Coleonema album*

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Abstract

Acetone and ethanolic leaf extracts of *Coleonema album* were screened for anti-inflammatory and antimycobacterial activity using the cyclooxygenase (COX-1 and COX-2) and broth micro-dilution assays against *Mycobacterium aurum* A+ respectively. In the cyclooxygenase assays, all extracts showed preferential activity against COX-2 with an IC_{50} value of 40 μg/ml for the acetone extract. The same extract had an IC_{50} value of 225 μg/ml against COX-1 with a COX-2/COX-1 selectivity ratio of 0.17. In the antimycobacterial test all extracts showed moderate activity against *M. aurum* with an MIC value of 3.1 mg/ml. The results suggest more in depth investigations into the anti-inflammatory compounds present in *C. album*.

Keywords: Anti-inflammatory; Antimycobacterial; *Coleonema album*; Rutaceae

Coleonema album (Thunb.) Bartl. & Wendl. is a shrub about 1 m tall with dense branching from the base. It grows along the coast in rock outcrops of the Table Mountain Sandstone Series. Its main population centre is in the Cape Peninsula. The plant is cultivated widely in gardens across South Africa (Williams, 1981).

During previous investigations into biological activities of *C. album*, antimicrobial effects, antioxidant activity and *in vitro* pharmacological action on smooth muscle were reported (Brader et al., 1997; Lis-Balchin et al., 2001; Lis-Balchin and Hart, 2002; Esterhuizen et al., 2006a,b). A large number of compounds had been isolated from *C. album* (phenolic acids, flavonoids, coumarins, prenylated coumarins and terpenoid compounds). Some of these compounds have activity against several inflammatory mediators (Brader et al., 1997; Silva et al. 2003; Chao et al., 2005; Esterhuizen et al., 2006a). Despite the fact that a tincture made from the plant and marketed as "Immunat" is widely used as a herbal remedy, there is no report on the anti-inflammatory activity of extracts from *C. album*. We report here on the inhibition of cyclooxygenase and antimycobacterial effects of ethanolic and acetone extracts of *C. album* shoots.

Plant material was collected and identified from Kleinmond (Western Cape) by C. Geldenhuys. A voucher specimen (Eldeen 11) is deposited in the Herbarium of The University of KwaZulu-Natal Pietermaritzburg. Shoots were collected and dried in an oven at 50 °C for five days. Extracts were made from powdered and non-powdered plant materials using acetone and ethanol (10 mg/ml). After sonication for 1 h, the extracts were filtered and concentrated to dryness under reduced pressure.

Anti-inflammatory activity of the plant extracts was determined using both the COX-1 and COX-2 assays. The basic protocol is the same for both assays, allowing a comparison of the inhibitory effects of the extracts on the two enzymes. The COX-1 bioassay was performed according to the method described by Jäger et al. (1996). The COX-2 assay described by Noreen et al. (1998) with slight modifications (Zschocke and Van Staden, 2000) was followed. In each test assay, four controls were run. Two were background in which the enzyme was inactivated with HCl before the addition of [^{14}C]arachidonic acid, and two were solvent blanks. Indomethacin was included in each test assay as a standard (5 μM for the COX-1 assay and 200 μM for the COX-2 assay). Both assays were performed in duplicate with double

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Table 1
Inhibition (%) of prostaglandin synthesis and IC_{50} values and minimum inhibitory concentration (MIC) values against *Mycobacterium aurum* A+ with powdered and non-powdered extracts of leaves of *Coleonema album*

Plant material analyzed	Solvent	COX-1 inhibition (%)						COX-2 inhibition (%)						COX-2/ COX-1 ratio	MIC values (mg/ml)	
		Concentrations tested (μg/ml)						Concentrations tested (μg/ml)								
		250	125	62.5	31.2	20	IC_{50}	250	125	62.5	31.2	20	IC_{50}		Extract	Ciprofloxacin (standard)
Powdered shoots	Ethanol	82	34	23	15	3	289	68	60	45	30	27	69	0.2	3.12	1.9 μg/ml
	Acetone	92	47	39	14	3	225	81	74	62	37	25	40	0.17	3.12	
Non-powdered shoots	Ethanol	78	41	34	16	3	222	65	55	37	31	28	96	0.4	3.12	
	Acetone	87	34	31	21	7	249	59	52	42	33	29	72	0.2	3.12	

Inhibition of prostaglandin and the MIC values were determined using the cyclooxygenase (COX-1 and COX-2) assays and the broth micro-dilution method respectively.
IC_{50} values recorded for the indomethacin (standard) against COX-1 and COX-2 in this study were 3.5 and 190 μM respectively.

determinations for each sample per assay. Percentage inhibition by the tested compound was calculated by comparing the amount of radioactivity present in the sample to that in the solvent blank. Data were fitted into Grafit version 5 software for estimation of IC_{50} values.

To determine antimycobacterial activity, Middlebrook 7H10 agar base (ref 453982) and Middlebrook 7H9 broth base (ref 454012) were used. The supplement OADC (oleic acid+albumin+dextrose+catalase) (Remel, USA) was added (10%) to both agar and broth media. A stock culture of *Mycobacterium aurum* A+ was obtained from the Microbiology Laboratory, Division of Pharmacology, University of Cape Town. The organism (stock number CIP 104482) was prepared as described in a Standard Operating Procedure outlined by Tracy Seaman of the Microbiology Laboratory. For determination of minimum inhibitory concentration (MIC) of *M. aurum* (SOP no. GL 2005/01) the broth micro-dilution method was used (Swenson et al., 1982). A multi-channel pipette was used to make a two fold serial dilution starting with a concentration of 25 mg/ml of the extracts and 62.5 μg/ml of ciprofloxacin used as standard control. The optical density (OD) of the 72 h liquid culture was adjusted to 0.125 at 550 nm. One hundred microliters of the diluted culture was added to every well of the microtitre plate excluding the wells of the first column that served as the medium control. The plates were loosely sealed in plastic bags to prevent drying out and incubated at 37 °C for 72 h. After incubation, 40 μl of 0.4 mg/ml solution of *p*-iodonitrotetrazolim salt (INT) was added to each well of the plate. The plates were left sealed in plastic packets overnight at 37 °C. The lowest concentration containing no indication of red colour as a result of INT was deemed to be the MIC.

Prostaglandin synthesis inhibition (%), IC_{50} values and COX-2/COX-1 ratios of powdered and non-powdered extracts of leaves of *C. album* are presented in Table 1. All powdered and non-powdered acetone and ethanolic extracts exhibited inhibitory activity against both COX-1 and COX-2 at the highest concentration tested (250 μg/ml). However, at a concentration of 125 μg/ml the extracts still showed good activity against COX-2 (more than 50%) but weak activity against COX-1 (less than 47%). The lowest IC_{50} was observed with the acetone powdered leaf extract against COX-2 (40 μg/ml). This extract had an IC_{50} of 225 μg/ml against COX-1. Ethanolic powdered and acetone non-powdered leaf extracts had a COX-2/COX-1 ratio of 0.2. The lowest ratio was observed with the acetone powdered extract (0.17). Based on IC_{50} values determined in this study, acetone extracts were more active than ethanolic extracts. Powdered materials also showed better activity than non-powdered materials. A number of chemical compounds have been isolated from *Coleonema* species, lignans (±)-sesamin and (±) prenylpiperitol the alkamide decadienoic acid isobutylamide, diterpene and phenylpropene evofolin-C-acetate (Brader et al., 1997). A series of prenylated coumarins and quercetin 3-galactoside were also isolated from the aerial parts of *C. album* (Esterhuizen et al., 2006a). Inhibition of COX enzymes in this study may well be due to the presence of these and/or similar compounds. The concept of IC_{50} of COX-2 to COX-1 ratio defines COX-2 selectivity by the plant extracts (Noreen et al., 1998). The lower the ratio (<1), the higher selectivity (Hawkey, 1999). All the extracts in this study showed a COX-2/COX-1 ratio less than one. Acetone powdered leaf extracts had a preferential activity against COX-2 with a selectivity ratio of 0.17. In view of the theory that selective inhibition of COX-2 with a lower ratio of COX-2/COX-1 will have potent anti-inflammatory activity, this finding is very interesting.

All the extracts in this study showed moderate activity against *M. aurum* A+. The minimum inhibitory concentration (MIC) obtained was 3.1 mg/ml (Table 1). This finding is in line with previously reported antimicrobial effects of acetone extracts (Esterhuizen et al., 2006a). The mechanism of action of antimycobacterials is still not very clear, however, Janin (2007) reported that the mycolic acids which are covalently linked to arabinogalactam, are components of the complex mycobacterial envelope. These long fatty acids are probably partly responsible for the antibacterial and antimycobacterial activity of the extracts. Further investigations into the compounds responsible for anti-inflammatory activity are warranted.

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